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# $Na^+/H^+$ exchange inhibitors reverse lactate-induced depression in postischaemic ventricular recovery

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1 By use of pharmacological approaches, the present study examined the hypothesis that the deleterious effect of lactate on postischaemic ventricular recovery may be mediated, at least in part, by enhanced activation of the $Na^+/H^+$ exchanger at the time of reperfusion.

2 Spontaneously beating isolated hearts of the rat were subjected to 15 min zero-flow global ischaemia followed by 30 min reperfusion. The effects of lactate (10, 20 or 40 mM) were studied by adding it 20 min before ischaemia whereas reperfusion was carried out with lactate-free buffer.

3 Pretreatment with 20 or 40 mM lactate significantly reduced postischaemic recovery of developed force to $17 \pm 3\%$ and $16 \pm 4\%$ of preischaemic values ($P < 0.05$) compared to a $78 \pm 4\%$ recovery in control hearts. Similarly, recovery in ventricular rate was significantly reduced to $34 \pm 7.6\%$ and $38 \pm 12\%$ with 20 and 40 mM lactate, respectively compared to $97.5 \pm 6.4\%$ recovery in control hearts. At a concentration of 10 mM, lactate was without effect on either force or ventricular rate recovery.

4 Coadministration of either of two $Na^+/H^+$ exchange inhibitors, amiloride (174 μM) or 5-N,N-hexamethylene amiloride (HMA, 1 μM) with lactate and inclusion of the two drugs during the first 5 min of reperfusion resulted in reversal of lactate-induced inhibition of force recovery with observed recoveries of $69 \pm 6.7\%$ and $64 \pm 5\%$ with amiloride and HMA, respectively. Similarly, recovery in ventricular rate was significantly enhanced to $92 \pm 10\%$ and $89 \pm 6\%$ with amiloride and HMA, respectively compared to $38 \pm 12\%$ recovery in control hearts.

5 In electrically paced hearts (5 Hz) subjected to 15 min ischaemia and 30 min reperfusion, 40 mM lactate significantly reduced force recovery to $36 \pm 5.5\%$ compared to $57 \pm 6\%$ in control hearts. In the presence of amiloride or HMA, force recovery in lactate-treated hearts was significantly increased to $68 \pm 16\%$ and $72 \pm 4.7\%$ of preischaemic values, respectively.

6 In spontaneously beating hearts, resting tension changes during both ischaemia and reperfusion were not statistically different between treatment groups. However, in paced hearts pretreated with 40 mM lactate the elevation in resting tension during the first 5 min of reperfusion, was significantly reduced by both amiloride and HMA.

7 Changes in functional recoveries produced by either lactate or $Na^+/H^+$ exchange inhibitors were unrelated to alterations in high energy phosphate depletion during ischaemia or to repletion of these compounds after 30 min reperfusion either in spontaneously beating or electrically paced hearts.

8 The results suggest that stimulated $Na^+/H^+$ exchange activation at reflow contributes, at least partially, to lactate-induced depression of postischaemic recovery.

**Keywords:** Rat heart; ischaemia; reperfusion; lactate; $Na^+/H^+$ exchange; contractile recovery; amiloride

## Introduction

Many factors are thought to regulate functional recovery of the reperfused myocardium following prolonged ischaemia including blood borne constituents such as neutrophils or endogenous myocardial principles such as defective energy metabolism or ionic cellular homeostatis. An interesting hypothesis proposed a number of years ago (Neely & Grotyohann, 1984) suggested that enhanced accumulation of glycolytic products, particularly lactate, at the end of the ischaemic period can contribute significantly to diminished recovery of ventricular function after reperfusion. The evidence for lactate involvement originated primarily from two observations; (1) prevention of lactate accumulation during ischaemia by glycogen depletion resulted in enhanced postischaemic recovery whereas, (2) exogenous lactate administration to isolated hearts significantly attenuated recovery (Neely & Grotyohann, 1984).

The mechanisms underlying lactate-induced depression in postischaemic recovery are unknown, nonetheless its inclusion in ischaemiamimetic solutions is required for the production of manifestations of tissue dysfunction in various experimental models (Ferrier *et al.*, 1985; Northover, 1987; 1989). One possibility is that lactate directly depresses contractility and thus recovery following reperfusion or inhibits energy substrate utilization (Tennant, 1935; Armiger *et al.*, 1974; Neely & Grotyohann, 1984). Alternatively, enhanced intracellular lactate accumulation and subsequent cellular acidification may contribute to diminished recovery. In this regard, it has been suggested that $Na^+/H^+$ exchange activation at the time of reflow would result in enhanced tissue injury as stimulation of this exchanger would elevate intracellular $Na^+$ concentrations and, subsequently, cellular $Ca^{2+}$ content via the $Na^+/Ca^{2+}$ exchanger (Lazdunski *et al.*, 1985). The possibility that $Na^+/H^+$ exchange inhibition provides a salutary influence on the reperfused ischaemic heart was supported by an initial report from the author's laboratory (Karmazyn, 1988), and that of others (Tani & Neely, 1989) that inhibitors of this exchanger significantly reduced reperfusion injury and enhanced functional recovery following ischaemia. Recently, we have reported that this protection is associated with a marked reduction in arrhythmias, preservation of cellular ultrastructure and reduction in mitochondrial injury (Duan & Karmazyn, 1992). The antiarrhythmic property of amiloride and amiloride analogues in reperfused myocardium has also been demonstrated by Dennis *et al.* (1990) in rat isolated hearts subjected to 15 min low-flow ischaemia followed by 2 min reperfusion. Moreover, the antiarrhythmic property of these drugs was similarly demonstrated in a canine model of coronary artery ligation and reperfusion (Duff *et al.*, 1991). In both of these studies, the antiarrhythmic effects were attributed to inhibition of the

$Na^+/H^+$ antiport (Dennis *et al.*, 1990; Duff *et al.*, 1991).

Because of the potential importance of the $Na^+/H^+$ exchanger to recovery following ischaemia, it was hypothesized that inhibition of postischaemic recovery by lactate could be mediated by an initial lactate-induced acid load followed by enhanced $Na^+/H^+$ exchange activation at the time of reflow. To examine this possibility indirectly, we evaluated the effect of amiloride (Frelin *et al.*, 1984; Simchowitz & Cragoe, 1986), a non specific $Na^+/H^+$ exchange inhibitor and 5-N,N-hexamethylene amiloride, an inhibitor of the exchanger demonstrating markedly greater specificity and potency than the parent compound (Simchowitz & Cragoe, 1986), on lactate-induced functional and metabolic changes in the ischaemic and reperfused rat heart.

## Methods

Male Sprague-Dawley rats weighing between 250 and 300 g were used in these studies. Animals were purchased from Charles River Canada Ltd. (St. Constant, Quebec, Canada). The animals were maintained in the Animal Care Facilities of the Faculty of Medicine, University of Western Ontario with free access to food and water in accordance with guidelines of the Canadian Council on Animal Care (Ottawa, Ontario).

For perfusion, the animals were killed by decapitation and the heart was rapidly excised, placed in a small crucible containing ice-cold Krebs-Henseleit buffer (composition below) and squeezed a few times with fingers to dislodge any clotted blood in the coronary vasculature. The heart was then mounted by the aorta on a stainless steel cannula and arranged for retrograde perfusion via the coronary arteries according to the Langendorff method as described in detail previously (Karmazyn, 1988; Watson & Karmazyn, 1991). The perfusion fluid was Krebs-Henseleit buffer (pH 7.4) of the following composition (in mM): NaCl 120, $NaHCO_3$ 20, KCl 4.63, $KH_2PO_4$ 1.17, $MgCl_2$ 1.2, $CaCl_2$ 1.25 and glucose 8 as substrate. The buffer was continuously gassed with a 95% $O_2$/5% $CO_2$ mixture. Flow rate was set at 10 ml $min^{-1}$ and controlled by a Watson-Marlow peristaltic pump. Contractile force (apicobasal displacement) was obtained by connecting a force displacement transducer (Grass FT.03) to the apex of the heart at an initial preload stretch of 2 g. For most experiments, hearts were allowed to contract spontaneously. In order to remove the contribution of rate to postischaemic recovery, some experiments were also done in which hearts were electrically stimulated at 5 Hz with a Grass S44 stimulator via platinum electrodes inserted into the ventricular epicardium. The entire system was regulated at 37°C. All recordings were obtained with a Grass polygraph.

The hearts were initially equilibrated for a 30 min perfusion period before induction of ischaemia. Where the effects of lactate were examined, sodium lactate (Sigma Chemical Co., St. Louis, MO, U.S.A.) was administered 20 min before ischaemia at a final concentration of either 10, 20 or 40 mM; for these experiments NaCl was reduced by equimolar concentrations, as appropriate. Only 40 mM lactate was examined in paced hearts. To test the effects of $Na^+/H^+$ exchange inhibitors, either amiloride (Sigma) dissolved in water after gentle warming (174 μM final concentration) or 5-N,N,-hexamethylene amiloride (HMA, 1 μM, Research Biochemicals Inc., Natick, MA, U.S.A.), dissolved in methanol (0.004% final buffer concentration) was added concomitantly with lactate. Initial experiments showed that neither of the two $Na^+/H^+$ exchange inhibitors or the HMA vehicle had a direct effect on cardiac function when added on their own.

Following the equilibration period, hearts were subjected to zero-flow ischaemia with or without a further 30 min reperfusion period. Zero-flow global ischaemia was initiated by turning off the perfusion pump for 15 min, care being taken to ensure temperature was maintained at 37°C during the ischaemic period. It should be emphasized that hearts initially treated with lactate were reperfused without lactate present in the buffer. However, when either amiloride or HMA was studied, these drugs were also present in the buffer for the first 5 min of reperfusion after which reperfusion was continued with drug-free buffer.

To obtain a full profile of energy metabolite content, hearts were clamped between tongs precooled in liquid nitrogen while on the perfusion cannula either immediately before the start of ischaemia, at the end of the 15 min ischaemic period or after 5 or 30 min of reperfusion. The frozen tissue was pulverized in liquid nitrogen and extracted with 6% perchloric acid for determination of high energy phosphate, adenine nucleotide or lactate content by use of enzymatic techniques as described previously (Bergmeyer, 1963; Watson & Karmazyn, 1991).

Statistical analyses were carried out by use of Analysis of Variance followed by Student-Newman-Keuls test to locate significant differences between treatment groups. Treatments were considered significant when $P<0.05$.

## Results

### *Basal function*

Table 1 demonstrates functional parameters of rat isolated hearts prior to initiation of ischaemia. No direct effect of 10 or 20 mM lactate on developed force or ventricular rate was observed whereas at 40 mM, lactate significantly depressed contractile force in the presence or absence of either amiloride or HMA. This cardiodepressant effect of 40 mM lactate was observed in both spontaneously beating or paced hearts, although with respect to the latter, a significant effect was seen only in the presence of HMA. Although the highest concentration of lactate exerted no effect on spontaneous ventricular rate, a significant negative chronotropic effect was observed in the presence of either amiloride or HMA (Table 1). No direct effect of lactate on resting tension was ever observed (not shown).

### *Tissue lactate contents*

Profiles of lactate content are shown in Table 2. Lactate values increased markedly following 15 min ischaemia and, expectedly, these values were further increased by lactate treatment in a concentration-dependent fashion. Thus, in hearts pretreated with 40 mM lactate, tissue lactate at the end of ischaemia was approximately 80% higher than seen in the absence of lactate. Elevations in lactate content were unaffected by either amiloride or HMA treatment. After reperfusion, lactate values rapidly (within 5 min) returned to values which were essentially similar to those seen in non-ischaemic hearts. This recovery was unaffected by either lactate pretreatment or by amiloride or HMA.

### *Effects of lactate on the ischaemic and reperfused heart*

Figure 1 shows the recovery of reperfused spontaneously contracting isolated hearts of the rat following a 15 min zero-flow ischaemic period, under control conditions or when pretreated with either 10, 20 or 40 mM lactate and reperfused with lactate-free buffer. In control hearts, force recovered to 78 ± 4% of preischaemic values after 30 min reperfusion which was not significantly affected by 10 mM lactate. However, 20 and 40 mM lactate significantly ($P<0.05$) attenuated force recovery to 17 ± 3% and 16 ± 4% of preischaemic values, respectively.

Recovery in ventricular rate was similar for control hearts and hearts treated with 10 mM lactate (96.5 ± 6.4% and 86 ± 7.6%, respectively, Figure 1). However, treatment with both 20 and 40 mM lactate significantly ($P<0.05$) depressed recovery of ventricular rate to 34 ± 7.6% and 38 ± 12% of preischaemic values, respectively (Figure 1). It should be noted that in hearts treated with lactate ($n=15$), 4 (26.6%)

Table 1 Function data before the initiation of ischaemia in spontaneously contracting and paced hearts

| *Treatment* | *Force (g)* | *Ventricular rate* (beats min$^{-1}$) |
|---|---|---|
| | Spontaneously contracting | |
| Control | 7.5 ± 0.4 | 329 ± 16 |
| Lactate 10 mM | 7.2 ± 0.6 | 309 ± 12 |
| Lactate 20 mM | 5.7 ± 1.0 | 311 ± 26 |
| Lactate 40 mM | 3.6 ± 0.5* | 321 ± 18 |
| Lactate 40 mM + amiloride | 3.5 ± 0.8* | 269 ± 23* |
| Lactate 40 mM + HMA | 4.0 ± 0.5* | 284 ± 13* |
| | Paced | |
| Control | 5.8 ± 0.5 | – |
| Lactate 40 mM | 3.9 ± 0.4 | – |
| Lactate 40 mM + amiloride | 3.7 ± 0.5 | – |
| Lactate 40 mM + HMA | 3.5 ± 0.3* | – |

Values indicate means ± s.e.mean of $n = 15$ except data for 10 mM lactate and paced hearts where $n = 5$. *$P < 0.05$ from control. HMA = hexamethylene amiloride. Data were obtained immediately before the initiation of ischaemia.

Table 2 Lactate content in control, ischaemic and reperfused hearts

| *Treatment* | *Buffer lactate concentration* (mM) | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 10 | 20 | 40 | 40 + Amiloride | 40 + HMA |
| | *Tissue lactate content* (μmol g$^{-1}$ dry wt.) | | | | | |
| 30 min normal perfusion | 8.3 ± 1.6 | 5.9 ± 2.5 | 19.8 ± 7.3 | 47.8 ± 16.5* | – | – |
| 15 min ischaemia | 97.8 ± 11.6 | 111.6 ± 23.6 | 136.9 ± 17.5 | 173.8 ± 21.5* | 197.6 ± 17.7* | 183.0 ± 27.4* |
| 5 min reperfusion | 5.9 ± 2.4 | 4.7 ± 0.9 | 4.9 ± 2.7 | 6.1 ± 1.1 | 4.61 ± 1.8 | 5.8 ± 1.7 |
| 30 min reperfusion | 7.8 ± 3.6 | 5.2 ± 0.7 | 6.3 ± 2.9 | 3.8 ± 1.2 | 5.9 ± 1.6 | 3.4 ± 2.1 |

Values indicate means ± s.e.mean of $n = 15$, except 5 min reperfusion and 10 mM lactate data where $n = 5$. *$P < 0.05$ from respective values obtained in the absence of exogenous lactate. HMA = hexamethylene amiloride. –; not done.

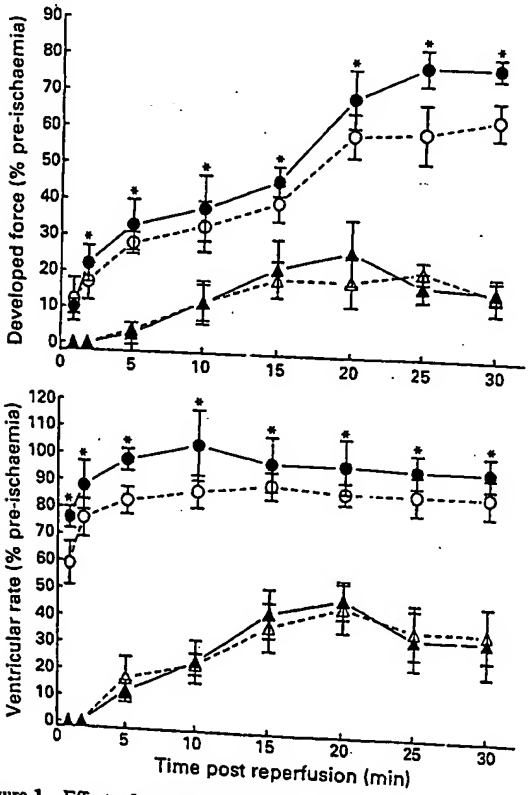


Figure 1 Effect of pretreatment with 10 (○), 20 (▲) or 40 (△) mM lactate on developed force and ventricular rate recovery in reperfused isolated spontaneously beating rat hearts following 15 min zero-flow ischaemia. See Methods for description of drug addition protocols. Values indicate means of $n = 15$ except for 10 mM lactate data where $n = 5$; s.e.mean shown by vertical bars. *$P < 0.05$ from values obtained with 20 or 40 mM lactate. (●) Control data.

and 6 (40%) totally failed to recover when treated with 20 or 40 mM lactate, respectively, whereas all control hearts or hearts treated with 10 mM lactate demonstrated functional recovery.

The effects of amiloride and HMA on diminished post-ischaemic recovery induced by either 20 or 40 mM lactate were examined. The results obtained with either inhibitor were similar irrespective of lactate concentration; thus, for conciseness results are presented only for those studies in which 40 mM lactate was used (Figure 2). As shown in Figure 2, both amiloride and HMA significantly improved recovery of force to 69 ± 6.7% and 64 ± 5% of preischaemic values, respectively, compared to 17 ± 2% seen with lactate alone. In addition, the 38 ± 12% recovery in ventricular rate after 30 min reperfusion was significantly increased to 92 ± 10% and 89 ± 6% in hearts treated with amiloride or HMA, respectively (Figure 2).

Because of the substantial effects of various treatments on ventricular rate, experiments were also done to assess the influence of 40 mM lactate and $Na^+/H^+$ exchange inhibitors in hearts in which ventricular rate was maintained by electrical pacing. As shown in Figure 3, control hearts subjected to this protocol recovered 57 ± 5.5% contractility, markedly lower than described above for spontaneously beating hearts. Nonetheless, lactate significantly ($P < 0.05$) depressed recovery to 36 ± 6% after 30 min reperfusion. Both amiloride and HMA improved recovery to 68 ± 16% and 72 ± 5%, respectively, values which were not different from control but significantly higher than those seen with 40 mM lactate alone (Figure 3).

Table 3 shows a summary of resting tension data in ischaemic and reperfused hearts and the influence of lactate. Under control conditions, resting tension was unaffected by ischaemia but was rapidly increased by reperfusion with a progressive return to close to preischaemic values thereafter. At all concentrations studied, lactate failed to alter significantly this response in hearts which were allowed to contract spontaneously. However, in paced hearts the eleva-

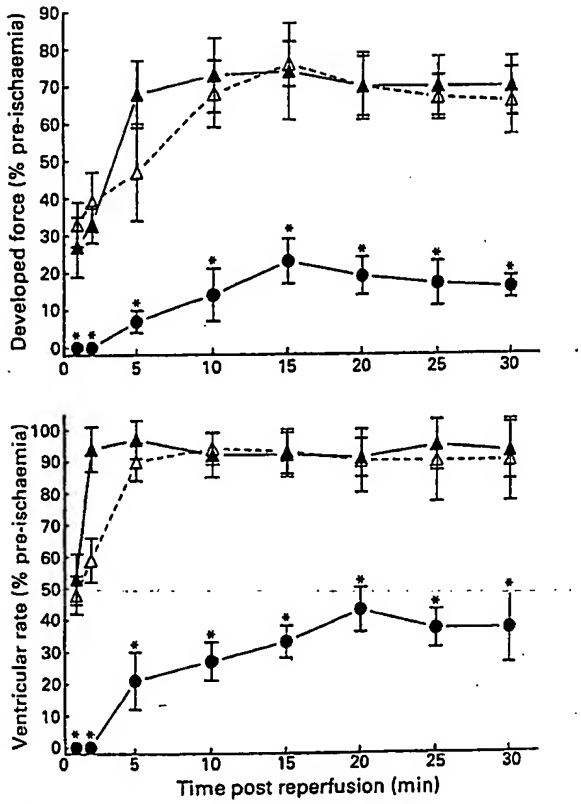


Figure 2 Effect of amiloride (▲) or 5-N,N-hexamethylene amiloride (△) on depression in postischaemic developed force and ventricular rate recovery produced by 40 mM lactate in reperfused isolated spontaneously beating rat hearts following 15 min zero-flow ischaemia. See Methods for description of drug addition protocols. Values indicate means of $n = 15$; s.e. mean shown by vertical bars. $^*P < 0.05$ from values obtained with lactate alone. (●) Effect of lactate alone.

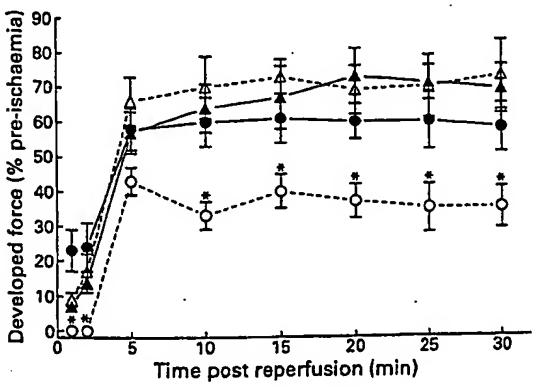


Figure 3 Effect of pretreatment with 40 mM lactate in the presence or absence of amiloride or 5-N,N-hexamethylene amiloride (HMA) on developed force recovery in reperfused isolated paced rat hearts following 15 min zero-flow ischaemia. See Methods for description of drug addition protocols. Values indicate means of $n = 5$; s.e. mean shown by vertical bars. $^*P < 0.05$ from other treatment groups except the first two points which indicate a significant difference from control group only. (●) Control; effect of lactate alone (○) and in the presence of amiloride (▲) or HMA (△).

tion in resting tension in the presence of 40 mM lactate was significantly attenuated by both amiloride and HMA (Table 3).

### *Profile of myocardial energy metabolism*

Table 4 shows a summary of changes in energy products in terms of high energy phosphates and adenine nucleotide contents under the various experimental conditions. Fifteen minutes of zero flow ischaemia resulted in a large and significant reduction in tissue high energy phosphates, particularly creatine phosphate (CrP). These changes were accompanied by increases in adenosine 5'-diphosphate (ADP) levels. The magnitude of these changes was unaffected by any treatment. Reperfusion for 30 min resulted in an incomplete recovery in high energy phosphate and adenine nucleotide contents. Interestingly, there were no significant differences in these values between any of the treatment groups studied, despite the substantial effects on contractile recovery produced by lactate pretreatment and $Na^+/H^+$ exchange inhibitors.

## Discussion

Myocardial ischaemia results in numerous biochemical aberations among which are the accumulation of various products derived from anaerobic glycolytic metabolism (Neely & Feuvray, 1981). It has been suggested that accumulation of these products during ischaemia may contribute to diminished functional recovery upon reperfusion (Neely & Grotyohann, 1984). Of these, lactate has been suggested to represent a particularly important factor in limiting recovery. This contention is supported by the finding that prevention of lactate accumulation through glycogen depletion results in improved recovery of isolated hearts following reperfusion whereas addition of exogenous lactate results in markedly diminished recovery (Neely & Grotyohann, 1984). Nevertheless, the involvement of lactate and approaches aimed at preventing its accumulation still remain controversial. For instance, with respect to lactate accumulation, it has been shown that preischaemic hypoxia and subsequent glycogen depletion in rabbit hearts does not prevent lactate accumulation during ischaemia and indeed actually results in diminished recovery after reperfusion (Lagerstrom *et al.*, 1988). Furthermore, it has recently been demonstrated that exogenous lactate (10 or 50 mM) failed to increase damage in rat cardiac myocytes subjected to anoxia and reoxygenation (Geisbuhler & Rovetto, 1990). The reasons for such apparent discrepancies still remain to be elucidated although they may be related to such factors as species or experimental model.

The present results are supportive of previous studies demonstrating deleterious effects of lactate in various models of myocardial ischaemia (Neely & Grotyohann, 1984; Ferrier *et al.*, 1985; Northover, 1987; 1989) which demonstrate that exogenous lactate, at concentrations similar to those used in the present study, can inhibit postischaemic recovery. It is important to emphasize that although lactate is a cardiodepressant on its own, the ability of lactate to depress recovery is most likely due to cellular mechanisms initiated by ischaemia and reperfusion since reflow was carried out in the absence of lactate in the perfusion medium. Indeed, as early as 5 min after reperfusion, lactate contents were similar in all treatment groups despite markedly different degrees of functional recovery. In addition, it should be emphasized that 20 mM lactate was equally effective in reducing postischaemic recovery in the absence of a significant direct negative inotropic influence. The inability of 10 mM lactate to depress recovery was surprising, although it may suggest that critically high tissue lactate levels must be achieved in order for the deleterious effect of lactate to be manifested.

Differences in depletion in high energy phosphate stores as a consequence of ischaemia or the degree of repletion of these compounds after reperfusion were also unlikely to represent contributing factors, since values did not differ between treatment groups under any of the conditions studied. This finding is in agreement with various other studies showing dissociation between depletion-repletion of high energy phosphates and postischaemic ventricular recovery under various experimental conditions (Higgins & Blackburn, 1984; Neely & Grotyohann, 1984; Karmazyn & Neely, 1989; Currie & Karmazyn, 1990; Murphy *et al.*, 1991). Not

Table 3 Effects of treatments on resting tension in ischaemic and reperfused hearts

| *Time* | Control | Lac 40 mM | *Treatment* Lac 40 mM + Amil | Lac 40 mM + HMA |
|---|---|---|---|---|
| | | | *Resting tension* (g) | |
| | | | *Spontaneously beating hearts* | |
| 0 min | 2.0 ± 0 | 2.6 ± 0.4 | 2.0 ± 0.3 | 2.0 ± 0.1 |
| 15 min isch | 2.0 ± 0 | 2.3 ± 0.5 | 2.0 ± 0 | 2.0 ± 0 |
| Reperfusion | | | | |
| 5 min | 4.0 ± 0.7 | 4.4 ± 1.1 | 2.6 ± 0.5 | 3.3 ± 0.5 |
| 10 min | 2.7 ± 0.4 | 3.7 ± 0.8 | 2.5 ± 0.5 | 3.2 ± 0.5 |
| 15 min | 2.4 ± 0.4 | 2.7 ± 0.6 | 2.2 ± 0.2 | 2.7 ± 0.3 |
| 20 min | 2.3 ± 0.3 | 2.5 ± 0.4 | 2.3 ± 0.1 | 2.2 ± 0.2 |
| 30 min | 2.4 ± 0.5 | 2.7 ± 0.8 | 2.0 ± 0 | 2.2 ± 0.2 |
| | | | *Paced hearts* | |
| 0 min | 2.1 ± 0.2 | 2.0 ± 0.3 | 2.0 ± 0.1 | 2.0 ± 0.2 |
| 15 min isch | 3.1 ± 0.4 | 4.3 ± 0.4 | 2.7 ± 0.4 | 2.1 ± 0.5 |
| Reperfusion | | | | |
| 5 min | 5.5 ± 0.9 | 7.1 ± 1.2 | 4.1 ± 0.3* | 3.6 ± 0.5* |
| 10 min | 3.9 ± 1.3 | 4.7 ± 0.8 | 2.9 ± 0.5 | 3.0 ± 0.4 |
| 15 min | 2.7 ± 0.5 | 3.6 ± 0.6 | 2.5 ± 0.6 | 2.5 ± 0.4 |
| 20 min | 2.5 ± 0.4 | 3.0 ± 0.3 | 2.7 ± 0.2 | 2.3 ± 0.4 |
| 30 min | 2.4 ± 0.4 | 3.0 ± 0.3 | 2.6 ± 0.3 | 2.3 ± 0.4 |

Values indicate means ± s.e.mean, $n = 15$ for spontaneously beating hearts and 5 for paced hearts. *$P < 0.05$ from values obtained with 40 mM lactate alone. Amil = amiloride, HMA = hexamethylene amiloride, Lac = lactate, isch = ischaemia. Zero min data refer to values immediately before ischaemia.

Table 4 High energy phosphate and adenine nucleotide contents in control, ischaemic and reperfused hearts

| *Treatment* | *Metabolite* (μmol $g^{-1}$ dry wt.) ATP | CrP | ADP | AMP |
|---|---|---|---|---|
| 30 min normal perfusion | | | | |
| Control | 22.4 ± 2.7 | 28.3 ± 3.9 | 7.3 ± 1.0 | 3.4 ± 0.5 |
| Lactate 10 mM | 24.6 ± 4.3 | 32.7 ± 4.0 | 6.8 ± 0.7 | 3.1 ± 0.6 |
| Lactate 20 mM | 23.7 ± 2.9 | 31.6 ± 1.0 | 7.0 ± 0.6 | 2.7 ± 0.6 |
| Lactate 40 mM | 23.3 ± 1.3 | 30.8 ± 5.2 | 6.6 ± 1.0 | 3.0 ± 0.7 |
| 15 min ischaemia | | | | |
| Control | 7.1 ± 1.6 | 3.1 ± 0.7 | 11.9 ± 2.0 | 1.6 ± 0.5 |
| Lactate 10 mM | 7.1 ± 0.9 | 2.7 ± 1.3 | 12.4 ± 3.3 | 2.4 ± 0.6 |
| Lactate 20 mM | 9.8 ± 2.1 | 4.0 ± 0.7 | 13.3 ± 0.8 | 1.9 ± 0.8 |
| Lactate 40 mM | 10.8 ± 3.8 | 4.2 ± 0.4 | 8.8 ± 1.0 | 5.3 ± 0.7 |
| Lactate 40 mM + amiloride | 11.9 ± 1.3 | 6.9 ± 1.8 | 10.3 ± 2.8 | 4.7 ± 0.8 |
| Lactate 40 mM + HMA | 12.1 ± 3.4 | 5.8 ± 1.5 | 9.9 ± 1.3 | 5.2 ± 0.9 |
| 5 min reperfusion | | | | |
| Control | 11.6 ± 1.5 | 9.8 ± 1.2 | 5.1 ± 0.6 | 2.5 ± 0.8 |
| Lactate 10 mM | 9.7 ± 1.3 | 8.7 ± 0.8 | 4.9 ± 1.1 | 3.0 ± 0.7 |
| Lactate 20 mM | 12.3 ± 1.8 | 13.6 ± 2.8 | 5.8 ± 1.2 | 3.1 ± 0.9 |
| Lactate 40 mM | 10.7 ± 2.3 | 11.8 ± 1.7 | 4.9 ± 0.8 | 2.7 ± 0.6 |
| Lactate 40 mM + amiloride | 13.5 ± 1.6 | 12.9 ± 1.3 | 5.7 ± 0.7 | 2.5 ± 0.3 |
| Lactate 40 mM + HMA | 12.2 ± 3.0 | 14.6 ± 1.8 | 5.8 ± 1.0 | 2.9 ± 0.7 |
| 30 min reperfusion | | | | |
| Control | 14.7 ± 3.1 | 19.8 ± 4.0 | 4.9 ± 0.8 | 2.8 ± 0.7 |
| Lactate 10 mM | 17.3 ± 2.9 | 19.0 ± 4.8 | 5.3 ± 1.0 | 3.1 ± 0.5 |
| Lactate 20 mM | 11.9 ± 2.7 | 15.3 ± 3.2 | 5.0 ± 0.9 | 2.6 ± 0.7 |
| Lactate 40 mM | 12.5 ± 3.1 | 14.7 ± 3.6 | 4.9 ± 1.0 | 2.5 ± 0.8 |
| Lactate 40 mM + amiloride | 13.4 ± 4.1 | 15.7 ± 3.0 | 4.5 ± 0.9 | 2.3 ± 1.0 |
| Lactate 40 mM + HMA | 12.9 ± 3.4 | 16.8 ± 5.7 | 3.9 ± 0.6 | 2.0 ± 0.6 |

Values indicate means ± s.e.mean of $n = 15$ except 10 mM lactate data where $n = 5$. HMA = hexamethylene amiloride.

unexpectedly therefore, the only significant metabolic consequence of lactate pretreatment that was measured in this study was the significantly elevated lactate content at the end of ischaemia in the absence or presence of $Na^+/H^+$ exchange inhibitors.

Previous studies from our laboratory have demonstrated a protective effect of amiloride against reperfusion injury (Karmazyn, 1988; Duan & Karmazyn, 1992). It has been shown that this protective effect of amiloride was associated with an inhibition of the early (2 min after reperfusion) rise in tissue $Na^+$ concentrations and the subsequent attenuation of $Ca^{2+}$ accumulation (Tani & Neely, 1989). In addition, these authors have shown that the protective effect of amiloride can be mimicked by attenuation of lactate accumulation at the end of ischaemia (Tani & Neely 1989). Thus, the importance of $Na^+/H^+$ to reperfusion injury appears to be adequately documented.

The present study was done to examine pharmacologically if $Na^+/H^+$ exchange contributes to lactate-induced depression in postischaemic recovery. Amiloride exerts numerous inhibitory effects on other cellular processes including the $Na^+/K^+$ ATPase and the $Na^+/Ca^{2+}$ exchange at concentrations substantially higher than those required to inhibit $Na^+/H^+$ exchange or that were used in the present study (Floreani *et*

*al.*, 1987). To strengthen the concept of $Na^+/H^+$ exchange involvement, we also tested the effects of HMA, a markedly more specific and potent inhibitor of $Na^+/H^+$ exchange (Simchowitz & Cragoe, 1986). The ability of both agents to potently reverse lactate-induced depression of ventricular recovery suggests that a potential mechanism for the latter may involve stimulation of $Na^+/H^+$ exchanger. For instance, cellular lactate uptake (Dennis *et al.*, 1984; Mann *et al.*, 1985) could result in increased intracellular acidification during ischaemia and, since the pH gradient represents a primary determinant of $Na^+/H^+$ exchange activation (Lazdunski *et al.*, 1985; Piwnica-Worms *et al.*, 1986), increased $Na^+/H^+$ exchange activation upon reperfusion. It is also interesting to note that the beneficial effects of both amiloride as well as HMA were observed even though the drug treatment was restricted only to the initial 5 min of reperfusion, suggesting that early events during reflow modulate recovery. Nonetheless, it should be noted that $pH_i$ was not measured in the present study and hence the degree of intracellular acidification following lactate preperfusion cannot be established. In addition, although tissue lactate content was measured, this represents total content found both intracellularly as well as interstitial space. Thus, the precise degree of lactate uptake into myocytes cannot be established with certainty. Taken together, an alternative explanation for the protective effects of amiloride or HMA may exist: for example that both drugs prevented ischaemia-induced changes in cellular homeostasis. For instance, it has been shown that amiloride can block the elevations in both $[Na^+]_i$ and $[Ca^{2+}]_i$ that occur during ischaemia in the absence of adenosine 5'-triphosphate (ATP) preservation resulting in improved postischaemic recovery (Murphy *et al.*, 1991). In the present study, neither amiloride nor HMA exerted significant beneficial effects when administered at the time of reflow (data now shown), a finding in agreement with a previous study by the author (Karmazyn, 1988) at least with respect to amiloride. Thus, the possibility exists that modulation of cellular events occurring during ischaemia represents an important constituent for the salutary effects of $Na^+/H^+$ exchange inhibitors. It is also noteworthy that the deleterious effects of exogenous lactate and the protective effects of amiloride or HMA were not restricted to developed force since lactate also produced diminished recovery in rate which was reversed by the $Na^+/H^+$ exchange inhibitors. The mechanism for the rate effects is uncertain particularly in an isolated heart preparation which precludes detailed investigations into cellular mechanisms, especially in terms of electrophysiological changes. It is interesting that intracellular acidification inhibits the calcium channel (Kaibara & Kameyama, 1988) and it could therefore be speculated that combined ischaemia plus exogenous lactate results in prolonged inhibition of pacemaker activity upon reflow. However, this possibility cannot explain the ability of either amiloride or HMA to reverse the effects of lactate on rate in the reperfused myocardium. Moreover, as only ventricular rate was measured, it cannot be stated with certainty whether reduced rate recovery represented effects on ventricular or atrial conduction. Mechanistic-based studies are currently being planned using myocyte preparations, where intracellular events such as ionic and electrophysiological changes could be precisely monitored.

Irrespective of precise mechanisms involved, the results of the present study add to the emerging body of evidence that $Na^+/H^+$ exchange inhibitors may represent an important approach towards the salvage of the ischaemic myocardium following reperfusion procedures.

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